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Chemical analysis of cacao residues in archaeological ceramics from North America: considerations of contamination, sample size and systematic controls

Dorothy K. Washburn [a,*], William N. Washburn [b,*], Petia A. Shipkova [c,1], Mary Ann Pelleymounter [d,2]

[a] American Section, Penn Museum, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Metabolic Disease, Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, NJ 08543, USA
[c] Analytical Chemistry, Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, NJ 08543, USA
[d] In Vivo Pharmacology Consulting, LLC, USA

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A B S T R A C T

We address the issue of contamination in sampling and storage procedures and the requirements of sample size and controls necessary to assay ceramic vessels for absorbed chemical residues. We focus our discussion on the detection and quantification of the three methylxanthines – caffeine, theobromine and theophylline as a means to infer whether Mississippian and Southwestern vessels had been used for the consumption of a stimulating drink made from the seeds of *Theobroma cacao*, a tree that grows in the Mesoamerican tropics. Our research detected two statistically differentiated concentration levels of methylxanthines on objects in museum storage: vessels with low levels of methylxanthines from airborne particulates that we attribute to environmental contamination, and vessels with significant higher levels of the methylxanthines that we attribute to the archaeological record reflecting prehistoric cacao consumption. We propose that cacao was imported into the American Midwest/Southeast during the Mississippian platform mound tradition AD 1000–1300 and into the American Southwest during the Chaco Great House tradition AD 900–1200 and the Hohokam platform mound tradition AD 1300–1400.

1. Introduction

Chemical residue analysis is increasingly used in archeology to infer vessel function, food preparation, consumption practices and activities in specific site locales as well as to provide information about regional interaction patterns and trade through time and space. Insights from these studies have enabled archaeologists to address the character of as well as continuities and changes in these activities in much greater detail (eg. reviews in Barnard and Eerkins, 2007; Budd et al., 1989; Heron and Evershed, 1993; Skibo, 2013).

We address the recovery and analysis of residues on ceramic vessels that can be found either as substances such as food adhering to the walls of ceramic vessels or as invisible chemical components of food and other materials that have been absorbed into the vessel walls. Residues can be from the plant or animal kingdoms. A number of different sampling procedures and analytical workups have been developed for the analysis of these different classes of residues (eg. Evershed et al., 1990; Hopkins and Armitage, 2012; Hurst et al., 1989). Readers should consult Evershed (2008a,b) for two excellent reviews of issues pertaining to analysis of absorbed residues.

Evershed and colleagues have pioneered the development and testing of protocols for the detection of lipids in archaeological vessels (eg. Charters et al., 1993; Dudd et al., 1999; Evershed, 1993; Evershed et al., 1995, 1999, 2002; Heron et al., 1991). We focus on the chemical analyses of residues containing alkaloids (see review by Rafferty, 2007), specifically on the detection of the three methylxanthines—theobromine, theophylline, and caffeine—found in *Theobroma cacao* (Fig. 1). The beans from this tropical tree were used to prepare a stimulating drink by the Maya and other pre-Hispanic Mesoamerican peoples (Ritchie, 1975; Weinberg and Beaker, 2001). To identify vessels containing this material, it is necessary not only to identify a biomarker appropriate to the substance of interest, in this case a methylxanthine

* Corresponding authors. Tel.: +1 609 737 7451.
E-mail addresses: DKWashburn@verizon.net (D.K. Washburn), WNWashburn@gmail.com (W.N. Washburn), Petia_Shipkova@BMS.com (P.A. Shipkova), pelleymounterm@Yahoo.com (M.A. Pelleymounter).
[1] Tel.: +1 609 818 3202.
[2] Tel.: +1 267 337 5282.

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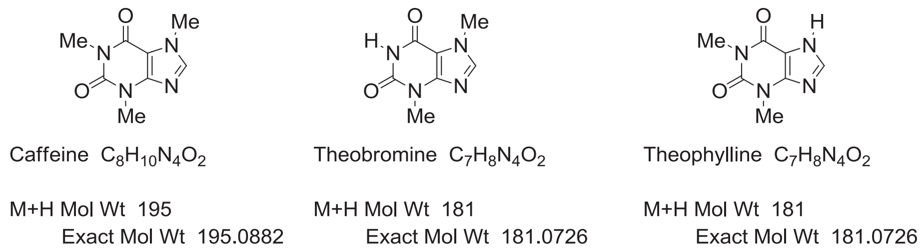

Fig. 1. Molecular structures and molecular weights for caffeine, theobromine and theophylline.

specific to cacao, but also to be certain that this biomarker reflects only archaeological activity and not contamination from other sources. Of three methylxanthines found in *T. cacao*, theobromine has been invoked as the biomarker for the presence of cacao in archaeological vessels (Hurst, 2006).

Ethnographic research indicates that the seeds and pulp of *T. cacao*, a tree native to the tropical humid coasts of Mexico and Central America, are used for various ritual and subsistence purposes. The beans are processed and often combined with other ingredients to make a simulating beverage (see articles in McNeil, 2006, also Prufer and Hurst, 2007; Soleri et al., 2013). Archaeological studies have found evidence that cacao was cultivated, consumed, and used as currency in pre-Hispanic Mesoamerica (Joyce and Henderson, 2007; Lentz et al., 1996; Millon, 1955; Sheets, 1992). A suite of ceramic vessels—cylinder jars, pitchers, and bowls were used to prepare, serve and consume cacao (Hall et al., 1990; Henderson et al., 2007; Hurst et al., 2002; Powis et al., 2007, 2011). Hieroglyphic inscriptions on vessels indicate their use for cacao (Gómez-Pompa et al., 1990; Stuart, 2006) as do painted pictorial images on Mayan cylinder jars, such as the "Princeton vase" that depicts women pouring cacao from one cylinder jar to another (Powis et al., 2002; Reents-Budet, 1994). These findings make it highly likely that the theobromine detected in these vessels was from cacao (McNeil et al., 2006, Table 11.1).

We address requirements of biomarkers, contamination issues, analytical methods, sample collection procedures, sample size, contamination controls and statistical analyses necessary to insure accurate chemical analysis of this class of compounds using liquid chromatography mass spectrometry (LCMS). We illustrate these requirements with an analysis of vessels from Mississippian period platform mounds in the American Midwest/Southeast as well as a reanalysis of our work from Ancestral Pueblo and Hohokam sites in the American Southwest. At issue is the nature of cacao trade and consumption in regions to the north of Mesoamerica. We argue that systematic analysis of robust samples can contribute to this debate.

2. Selection of appropriate biomarkers

Biomarkers must be unique to the compound of interest. Proper selection of a biomarker that will provide discriminatory information prevents faulty conclusions. Results may be confounded if the biomarker compound is present in more than the plant being targeted. It is essential to know all sources of a potential biomarker that are native to an area before drawing conclusions concerning its presence or absence in the analyzed samples. For example, users may have processed two different native plants each containing the biomarker of interest for totally diverse purposes in the same vessel. The risk of incorrect inferences for vessel function may be further heightened since vessels often have multiple uses over their use life. All-purpose jar and bowl forms are frequently used for dry and wet food storage as well as for preparation and cooking activities that mix both plant and animal products. Finally, special caution should be exercised testing sherds rather than whole vessels. In their new use life, the size and shape of sherds may have been suitable for use as scoops, scrapers and other purposes during which they came in contact with and absorbed residues unrelated to the original activities that took place when the vessels were whole. These different activities and substances in vessels over their use life may confound efforts to isolate diagnostic compounds indicative of the substance of interest.

Theobromine has been used as the biomarker of choice to detect cacao. In our studies of the ceramics from Pueblo Bonito and Los Muertos we were able to assert that the theobromine we detected was from *T. cacao* because plant databases did not list any other plant native to the American Southwest that contained theobromine (Washburn et al., 2011, p. 1638). However, in the Southeast, *Ilex vomitoria*, a holly that grows along the Gulf Coast also contains the methylxanthines caffeine and theobromine. Leaves from this holly were roasted and steeped in hot water by the historic Indians of the Southeast to make a tea called Black Drink that was used as a social beverage, medicine and emetic (Hudson, 1979). For this reason, it is not possible to use the presence of theobromine to distinguish between cacao and Black Drink.

To resolve this dilemma, Crown et al. (2012) endeavored to use ursolic acid as a biomarker to identify Black Drink in beakers from Cahokia, a very large platform mound complex near St Louis, MO. However, her analysis is fatally flawed since ursolic acid is a common phytochemical present in many species of fruits and berries native to the Southeast and Midwest (http://www.ars-grun.gov/duke). Scarry (2003, Table 3.1) reviews the fruit bearing trees and plants available to native peoples in these areas. Smith (2011, p. 840) reports that early travelers noted these plants flourishing adjacent to old Indian settlements. Many of these plants—black haw, grape, plum, bramble and elderberry—were found in flotation studies from Cahokia (Pauketat et al., 2002), the main site that contained the beaker sherds tested by Crown. Similarly, wild cherry, wild grape and dogwood were found at the nearby BBB motor site (Johannessen, 1984a; Whalley, 1984). In fact, Johannessen noted that fleshy fruits and berries (grape, blackberry, raspberry, elderberry and black haw) are present throughout the sites in the American Bottom region from the Late Archaic through the Woodland and Mississippian periods (Johannessen, 1984b). Harn (1980, p. 8) noted these plants along the central Illinois River valley. Consequently, there is a very high possibility that the Cahokia beakers and beakers from the other sites in the area tested by Crown may have been exposed to these plants containing ursolic acid. Thus, in this situation, invoking ursolic acid as the biomarker to distinguish Black Drink from cacao is invalid.

3. Contamination

Contamination is a primary concern for residue analysis. There are many potential opportunities for contaminants to confound residue analyses of archaeological materials. These range from introduction by the original users, introduction by microorganisms, absorption from contaminated ground water, and introduction by

human activity during the process of excavation, artifact cleaning, analysis and storage.

Contamination of buried objects can arise either by microbial transformations or through absorption of the biomarker from the environment. Compounds dissolved in the ground water, for example theobromine or caffeine, may be absorbed in the clay matrix of the vessels. Contamination by microbial metabolism to generate additional amounts of the compound of interest is best assessed by analyzing for other metabolites that would also arise if microbial activity was involved. For example, one pathway for bacterial metabolism of caffeine results in successive stepwise replacement of the three methyls to initially generate a mixture of theobromine and paraxanthine which are further converted to 7-methylxanthine which is then converted to xanthine. Our analysis of the extracts from both the control samples of dust as well as from the Southwest and Southeast vessels detected no evidence of paraxanthine. Failure to detect paraxanthine reduces the possibility that caffeine metabolism contributed to the amount of theobromine detected (MetaCyc, http://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-6538; Yu et al., 2009).

Regarding the preservation or degradation of alkaloids in soils, experimental studies indicate that caffeine was still detected by GC/MS in residues extracted from experimentally produced sherds impregnated with Black Drink that had been buried under different soil conditions for two months (Reber and Kerr, 2012). Similarly, Hopkins and Armitage (2012) report experiments where they soaked new terra cotta containers with slurrys of *T. cacao* for 24 h and then broke the vessels into sherds which they buried for up to six months. After excavation the sherds were washed as per standard field treatment and then scrubbed in the lab to remove remaining soil particles. Powdered samples of the vessels were found by DART-MS to contain both theobromine and caffeine.

Much less attention has been paid to the possibility of contamination *after* the ceramics have been excavated, that is, during the process of recovery in the field, while they are being analyzed in laboratories and, ultimately, under the conditions in which they are being stored in museums. Although best practices would minimize routine contamination with food or drink, chance contamination of a vessel would be readily apparent in a large sample since a drop of coffee or piece of chocolate would produce atypically elevated levels of the methylxanthines. Another possible source of contamination in research and storage environments is from airborne particulate matter. Morawski and Salthammer (2006) review potential sources of airborne contaminants. Museums have long considered the problem of destructive airborne contaminants resulting from soot, smog and other airborne industrial wastes entering museum environments in the public areas. A number of studies have assessed types, concentration, deposition time frame and damage from airborne particulates in museums (eg. Berland, 1993; Camuffo et al., 2001; Ligocki et al., 1993).

We focus here on a particular problem for investigators employing certain biomarkers such as one of the methylxanthines. Due to the popularity of coffee, tea, colas and chocolate in modern society, methylxanthines are pervasive in the environment. The process of roasting coffee (Partee, 1964) creates dust from the green beans and chaff from the skin of the beans that contribute airborne particulate matter. Further processing of the roasted coffee for powdered coffee emits still more particulate matter during the drying process. The reports of measurable airborne particulates from grinding and roasting coffee beans in major US cities (Dong et al., 1977a,b; Faith, 1977; Tabor et al., 1957) prompted us to examine the dust in museum storage environments for methylxanthines due to the extensive consumption of coffee, tea, chocolate, and other products containing these methylxanthines. We emphasize that it is solid particulate matter not airborne aroma droplets that are the source of contamination since studies of the aroma constituents of coffee did not detect caffeine (Grosch et al., 2000). The fact is that the 235 °C melting point of caffeine means that the vapor pressure is so exceeding low that it will not volatilize.

The research reported here is the first to show that caffeine and related methylxanthines are one component of the particulate matter that has settled as dust on artifacts in museums and research centers. Our control studies from six museums in six different cities and towns reveal a low level of theobromine and theophylline, but higher and more variable levels of caffeine in the dust on storage cabinets and shelves where the ceramics were stored. We discuss how contamination from airborne sources may be a confounding issue for different methods of sample collection. In particular our water wash collection approach is subject to this contamination since any particulate matter in the air that settled in the vessels would be captured in the water washes. However, we note that alternative procedures that analyze residues scraped from the ceramic surface will also collect these same airborne contaminants. Finally, there are no reported control studies that have tested whether surface scraping or burring will remove this airborne contamination.

4. Analytical methods

Residue analysis is critically dependent on the execution of a proper set of experimental conditions that will yield reliable results, establish a measure of the assay's sensitivity, contribute a perspective regarding the incidence of false positives and false negatives, and address the issue of contamination. It is important to understand the reliability of the analytical method. Analytical standards of the compound of interest (biomarker) and of other compounds that could confound data interpretation should be employed to establish the appropriate analytical procedures in order to achieve adequate resolution and quantification of these substances. A calibration curve to quantify the analyte (compound of interest) present in a set of samples should be generated, employing freshly prepared solutions spanning the projected concentration range. This will establish the sensitivity of the analytical equipment for analyses run on that particular day. Analyses of standards and samples should be run in duplicate to insure reproducibility. Artificial samples spiked with the analyte of interest should be run to provide a measure of the recovery efficacy under the same conditions used to extract the residue from the archaeological vessels.

The following describes the analytical instrumentation and procedures we employed to assay the residues collected for our studies of Midwest/Southeast and Southwest ceramics. All samples and standards were analyzed using a hybrid LTQ-Orbitrap mass spectrometer (thermo Scientific, San Jose, CA) interfaced with an Acquity UPLC (Waters, Milford, MA). An Acquity BEHC18, 50 mm, 1.7u UPLC column, kept at 65 °C, was used with a 0.6 mL/min flow rate. Mobile phase A was water, 0.1% formic acid and mobile phase B was 98.2 acetonitrile:water, 0.1% formic acid. The chromatographic conditions which provided baseline resolution of the three methylxanthines—theobromine, caffeine, and theophylline—included a 1 min hold at 100%A followed by a ramp to 100%B at 5 min. After a 1 min hold at 100%B, the column was equilibrated at the initial conditions for an additional minute. Accurate mass data (within 5 ppm) were collected at 15k resolution from 100 to 1000 Da. A calibration curve spanning 9–1100 ng/mL was generated using four freshly prepared standard solutions of each methylxanthine each time a set of samples was analyzed by LC/MS (Fig. 2). All analyses were run in duplicate; blanks of de-ionized were always included. In addition dummy samples were prepared by processing de-

ionized water in the same fashion as the aqueous vessel washes. Neither the blanks nor the dummy samples contained any methylxanthines.

5. Sampling collection

It is necessary to develop a consistent vessel sampling procedure that take into account conservation requirements that allow or prevent destruction of the sherd or vessel as well as the nature of the vessel wall matrix and surface treatment that affects whether the compounds adhere to the surface and/or are absorbed in the walls.

The prevailing method for collecting samples of absorbed residues involves destructive analysis. For some analyses this has entailed scraping actual residues from the interior of the vessel (Grant, 2006). For vessels without visible residue, investigator descriptions of sampling procedures vary but all involve some cleaning of the surface before collecting and pulverizing the sherd and extracting material from the pulverized powder. For example, samples from the Maya spouted vessels that had no visible residues on the interior surfaces were collected by lightly scraping the interior surface of each vessel (sample ranging from 1 to 7 g) (Powis et al., 2002; p. 97). The San Lorenzo sample was collected by lightly rubbing the interior of the sherd or vessel using fine-grained sandpaper to collect the material that had penetrated the clay (Powis et al., 2011; p. 8595). In both cases the clay powder that resulted from this abrasion was analyzed for methylxanthine content. For the analysis of the pitcher and cylinder jar base sherds from the Pueblo Bonito trash, the interior surface of the sherd was "burred" before the sherds were pulverized (Crown and Hurst, 2009; pp. 2110–2111, Crown et al., 2012; p. 13945). Despite the fact that the material scraped was subject to surface dust contamination, no controls have been reported that demonstrate that surface scraping, rubbing or burring removes surface contamination.

In our previous analyses of vessels from the Southwest as well as in the present analysis of vessels from the Midwest and Southeast, samples were collected from whole vessels using a water wash collection procedure that takes advantage of the water solubility of theobromine. Partial or whole vessels can be sampled without adverse conservation damage by pouring 30–40 mL of de-ionized water into a vessel. Using a pipette, the sides of the vessel were washed down with the water for ~5 min. The water was transferred by pipette to a vial. After standing for 24 h to allow the sediment to settle, the solution was concentrated under vacuum to ~0.5–1 mL. The concentrated liquid was then centrifuged 20 min at 13,500 × g to remove suspended particulates. After transfer of the clear supernatant to a clean tube, the process was repeated prior to mass spectral analysis. We established that the water wash sample collection procedure replicated Hurst's findings of theobromine in two Guatemalan Maya vessels at the University of Pennsylvania: a cylindrical jar from a burial cave in the Senahu district of Guatemala (NA 10835) and a bowl from the site of Chama (NA 11216) (Grant, 2006). Hurst obtained his samples by scraping residues from the interior sides of the vessels—a process that would also capture any airborne dust that had settled on the residues. However, for these two vessels the amounts of theobromine found by both methods were well above the cutoff values for theobromine introduced by airborne contamination.

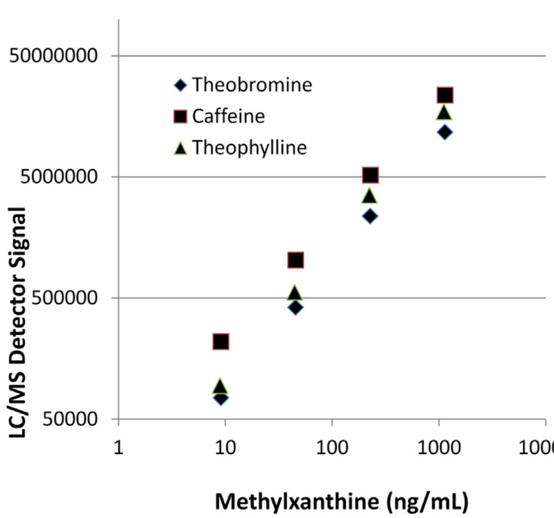

Fig. 2. Calibration curves for LC/MS response for theobromine ($y = 10365x - 18\ 267$), theophylline ($y = 15\ 539x - 42\ 956$) and caffeine ($y = 20\ 613x + 1\ 75\ 973$); $r^2 > 0.99$.

6. Sample size

Many residue analyses have reported the presence of a substance by sampling just a few objects. While such work may detect the presence of a given material at an earlier time or in a new region where it was previously unknown, presence/absence discoveries have very limited value in addressing the complex questions of human behavior being asked by archaeologists today. More importantly, analysis of one or two samples exposes the analyst to false positives or false negatives. Statistical analyses of robust samples should be employed to assess the significance of the results and to address the possibility of contamination. For example, if large samples are analyzed, then it will be possible to statistically differentiate residues containing the same biomarkers. Although both *T. cacao* and *I. vomitoria* contain theobromine, the two species differ with respect to amounts of the two other methylxanthines. *I. vomitoria* contains more caffeine than theobromine but no theophylline. In contrast, *T. cacao* contains more theobromine than either caffeine or theophylline.

7. Contamination controls

We ran several types of controls to determine whether contamination was an issue. In our first studies of Southwestern Chaco and Hohokam ceramics (Washburn et al., 2011) and Southeast ceramics, we detected methylxanthines in greater than 60% of the vessels. Given the suggestion that our water wash method may also be recovering contaminating methylxanthines from sources not related the archaeological record, we performed two kinds of tests. First we tested vessels from regions and time periods that are not known to have been associated with materials containing methylxanthines. Second we sampled the museum storage spaces in which the vessels were stored (Tables 1 and 2).

For the temporal controls, we analyzed water washes of 11 vessels, such as Neolithic beakers from Germany, that represented cultural activity well prior to the use of methylxanthine-containing native plants. For the geographical controls, we sampled 5 ceramic containers and stone lamps from areas of the world not thought to have had access to cacao—Alaskan sites dating to the Old Bering Sea period excavated by Henry B. Collins and James A. Ford in 1929. All three methylxanthines were present in all of the vessels from these temporal and geographical controls (Table 1). The measured theobromine concentration ranged from 1.4 to 32.7 ng/mL; the mean concentration was 14.3 with a standard deviation of 12.3 ng/mL. Caffeine and theophylline were detected in values ranging from 0.4 to 97 and 1.2 to 15.8 respectively, with mean values of 25.2 ± 26.4 and 5.2 ± 4.0 ng/mL respectively. These results

Table 1
Temporal and Geographical control vessel samples analyzed. $N = 16$.

Sample #	Catalog #	Vessel form	Museum	Site/provenience	Date	Theobromine	Caffeine	Theophylline
C1	7879	Cup	Penn	Switzerland, Lake Dwellings	Middle Bronze Age	2.28	0.35	1.77
C2	29-29-304	Cup	Penn	Bohemia, Liboc	Neolithic	21.73	69.39	6.01
C3	29-29-1142	Bowl	Penn	Bohemia, Bubenec	Bronze Age III	44.1	96.66	9
C4	65-25-116	Bowl	Penn	Bohemia, Drevice	Bronze Age III	12.17	16.73	3.68
C5	7880	Amphora	Penn	Switzerland, Lake Dwellings	Middle Bronze Age	7.06	15.74	2.36
C6	65-25-23	Amphora	Penn	Poland, Poznan	Early Iron Age, Urnfield	22.09	18.02	3.98
C7	7881	Amphora	Penn	Switzerland, Lake Dwellings	Middle Bronze Age	4.62	3.52	1.76
C8	A342736	Stone lamp	Smithsonian	Alaska, Gambell	Old Bering Sea	7.62	29.71	6.71
C9	A342810	Stone lamp	Smithsonian	Alaska, Punuk Is.	Old Bering Sea	2.94	4.2	2.33
C10	A342746	Bowl	Smithsonian	Alaska, Punuk Is.	Old Bering Sea	32.72	176.08	15.76
C12	A400348	Vase	Smithsonian	Alaska, Point Barrow	Old Bering Sea	2.94	10.77	4.65
C13	A342685	Bowl	Smithsonian	Alaska, Cape Kialegak	Old Bering Sea	1.38	0.98	1.22
C14	59-36-40/7676	Neck jar	Peabody	France, Chalons sur Marne	Iron Age, La Tene II	9.2	19.5	2.7
C15	71-19-40/5025	Neck jar	Peabody	Switzerland, Lake Neuchatel	Iron Age, Hallstadt A	15.5	31.5	9.5
C16	38-64-40/4318	Beaker	Peabody	Germany, Weimar	Late Neolithic, Bell Beaker	17.7	26.3	2.9
C17	39-63-40/5476	Neck jar	Peabody	Poland, Poznan	Late Bronze Age, Lusatian	25.2	33.9	9.5

indicated that a source for the methylxanthines other than cacao must exist.

We tested whether airborne particulates were a source of vessel contamination in the museum storage environment by analyzing the dust on the shelves where the vessels were stored. We took dust samples from inside and outside storage cabinets, on open shelving, and in different rooms and locations in the six museums that housed the vessels we studied: American Museum of Natural History, Smithsonian Institution Suitland storage facility, Peabody Museum at Harvard University, Penn Museum at University of Pennsylvania, Illinois State Museum Springfield Research facility and the Dickson Mounds Museum. These samples will be referred to as the dust samples (Table 2).

To collect each dust sample a cotton ball dampened with de-ionized distilled water was used to swab an area of ~36 cm^2, an area that would approximate or exceed the orifice of most vessels sampled. After wiping the area, the cotton balls were placed in clean screw top glass vials. Sample preparation for analysis entailed addition of 10 mL of de-ionized water prior to sonication of the vials for 30 min at 40 °C to promote dissolution of any methylxanthine. Using the same procedure described for washes of the pottery vessels, the water was removed by pipette and concentrated to 0.5–1 mL prior to centrifugation and LC/MS analysis. Blanks included similarly processed clean cotton balls. Methylxanthines were detected in all dust samples but not in the cotton ball controls. Theobromine values ranged from 1.3 to 31.4 ng/mL; the mean concentration was 12.1 ± 8.3 ng/mL. Caffeine and theophylline values ranged from 5.7 to 298.3 and 0.9 to 3.9 respectively; the mean values were 69.7 ± 83.1 and 6.2 ± 3.90 ng/mL respectively (Table 2). These results indicated that artifacts stored in museums are subject to airborne methylxanthine contamination. It should be noted that the caffeine levels show much more variation than the theobromine and theophylline levels in the dust samples from all six museums.

8. Statistical analysis

The results from both the temporal and geographical samples and the dust samples unambiguously demonstrate that methyl-xanthines are present in low but pervasive quantities due airborne

Table 2
Dust control storage area samples analyzed. $N = 25$.

Sample #	Museum	Storage location	Theobromine	Caffeine	Theophylline
D1	Penn	Top metal cabinet where European pots tested stored	1.26	10.44	0.8
D2	Penn	Top metal cabinet where SW pots tested stored	5.9	5.68	1.75
D3	Penn	Metal shelf where SW pots tested stored	2.61	5.74	1.95
D4	Dickson Mds	Wood drawer where pots tested stored	2.7	16.5	0.9
D5	Dickson Mds	Top wood cabinet where pots tested stored	9.7	16.7	2.2
D6	Ill St Mus	Metal shelf of cabinet where pots tested stored	14.9	939.4[a]	8
D7	Ill St Mus	Top metal cabinet where pots tested stored	27.4	908.3[a]	11.2
D8	Smithsonian	Top metal cabinet where SW pots tested stored	8.7	79.1	6.3
D9	Smithsonian	Top metal cabinet where Arctic pots tested stored	10.3	91.3	8.2
D10	Peabody	Ethafoam on shelf where Neolithic pot tested stored	15.1	31.3	5.7
D11	Peabody	Ethafoam on shelf where Southeast pots tested stored	22.2	26.3	9
D12	Peabody	Metal shelf where Bronze Age pots tested stored	31.4	13.6	9.3
D13	Peabody	Ethafoam on shelf where Bonito pots tested stored	11.9	15	3
D14	Peabody	Ethafoam on shelf where Los Muertos pots tested stored	25.1	39.7	7
D15	Peabody	Ethafoam on shelf where B/W SW pots tested stored	9.7	13.6	2.9
D16	Peabody	Ethafoam on drawer where Abajo pots tested stored	9.5	13.5	2.8
D18	Peabody	Ethafoam on drawer where Abajo pots tested stored	8.4	15.2	2.8
D19	Peabody	Ethafoam on shelf where B/W SW pots tested stored	16.2	29.6	3.9
D20	Peabody	Metal shelf where Turner Mound cylinder jar tested stored	17.3	34.3	5.7
D21	AMNH	Top metal cabinet in room where SW pots tested stored	11.2	298.3	13.9
D22	AMNH	Metal shelf inside cabinet where SW pots tested stored	4.3	227.2	9.5
D23	AMNH	Old metal shelving adjacent to SW storage cabinets	4.3	185.7	9.3
D24	AMNH	Top of metal cabinet in room where Chaco pots tested stored	7.5	168.5	10.6
D25	AMNH	Metal shelf inside cabinet where Chaco pots tested stored	2.1	90.4	4.6
D26	AMNH	Top wood cabinet in entryway adjacent to door to public area	22.5	174.4	12.8

[a] Values not used.

contamination. To exclude airborne contamination being the sole source, ANOVA statistical analysis was employed to ascertain whether the population distribution of the methylxanthine concentrations was the same for the archaeological vessel set versus either the dust or geographical controls. One way analysis of variance (ANOVA) enabled us to compare methylxanthine levels in three groups: 1) archaeological samples from the American Midwest/Southeast and Southwest, 2) samples from vessels originating from unrelated geographical areas and temporal periods, and 3) samples of dust taken from museums where the archaeological samples were stored. ANOVA estimates the probability that these levels are the same in all three groups. If there is less than a 5% probability that the methylxanthine levels are the same, there is a 95% chance that the methylxanthine levels are different among these groups. This suggests a systematic difference between methylxanthine levels from the archaeological samples and the methylxanthine levels due to other non-archaeological sources in the environment.

We conducted separate ANOVA analyses for each methylxanthine, comparing levels in the Mississippian and Southwest vessels to the levels in the dust samples and the geographical controls. In order for the data to satisfy the equality of variance requirement of parametric statistical testing, log transformation was utilized. Therefore, all statistical comparisons were conducted with log transformed data. Separate ANOVA analyses were conducted for each site. Post-hoc group comparisons were conducted using Fishers Least Significant Difference tests. The data were back-transformed to their original numbers for graphing purposes.

9. Data bases analyzed

The nature of the relationship between the cultural traditions of Mesoamerica and the American Southwest and Midwest/Southeast has long been debated (reviews in Lekson, 2006; White and Weinstein, 2008). Citing near absence of objects made in Mesoamerica, many archaeologists model the rise of the great house and platform mound centers in these areas as an indigenous, autochthonous process. The absence of quantities of artifacts made in Mesoamerica has been used to argue that any contact with Mesoamerica was infrequent and probably indirect. We sought to examine these assumptions and assertions with chemical assays of the contents of a robust sample of vessels that tested for food compounds that had to be imported from Mesoamerica. To this end, we sought to analyze a suite of evidence that correlated the presence of non-local vessel forms decorated with non-local design systems with chemical evidence that they contained a non-local beverage, cacao.

9.1. Midwest/Southeast analysis

In the Middle Mississippi region archaeologists have focused on the development of and contacts between large sites such as Cahokia and small, hinterland sites in the Late Woodland and Mississippian periods (see reviews in Emerson and Lewis, 1991; Kelly, 1991; Milner, 1990; Steadman, 1998; see also Alt, 2002; Holt, 2009; Pauketat, 1998, 2002). The Mississippian Tradition describes a period of large platform mound cities and associated smaller peripheral mound and burial mound sites along the Mississippi River and its tributaries. Cahokia is the largest complex, consisting of 120 monumental solid earthen platform mounds and plazas covering 6 square miles on the eastern side of the Mississippi River opposite present day St Louis. It is surrounded by smaller mound centers in the immediate American Bottom region as well as more peripheral centers, such as the Emmons Site and the Dickson Mound site that we studied along the Illinois River. The shell-tempered vessel forms tested associated with this platform mound building period include cylindrically shaped jars, effigy bowls, mug-like handled beakers also known as Tippets bean pots, water bottles with tall necks and jars with pronounced angled shoulders called Rami Incised. These ceramics span the Mississippian tradition from the late Woodland/Emergent Mississippian Sepo phases (AD 1000) through the Eveland/Stirling phase (AD 1100–1200) into the decline of Cahokia during the Moorehead (AD 1200–1275) and later Larson phases (AD 1300) (Steadman, 2001, Table 1).

Here we suggest not only that we can learn more definitively whether cacao was consumed in these Midwest/Southeast platform mound centers with chemical analysis, but also that we can use this evidence to assess other issues, such as the center/periphery relationship. For example, testing of large samples from different site types enables us to as the question—if cacao consumption was ritually consumed by the elites, did this status group live only at the large centers, or did they also live in the peripheral communities? Our chemical residue evidence of the unique vessel forms present at Cahokia as well as at the hinterland sites indicates that Mesoamerican practices, such as cacao consumption, shaped the ritual activities at both the platform mound centers and their associated hinterland complexes.

Just as among the Maya and other Mesoamerican state societies, as well as among the inhabitants of the great houses at Chaco (Crown and Hurst, 2009; Washburn et al., 2011) the main indication that cacao consumption might be taking place was the presence of a cylinder jar at Cahokia (Fig. 3). While the cylindrical jar form is rare in the American Southwest, being present only in the great house pueblos in Chaco Canyon, northwest New Mexico, in the American Southeast, this jar form appears more frequently in the lower Mississippi River valley/Gulf coast area. It is found from Weeden Island, Florida (Griffin, 1952, Fig. 180G) to the Caddo area of east Texas and southwest Arkansas (Newell and Krieger, 1949, Figs. 39, 41–42). Globular and straight-sided cylindrical forms appeared during the Troyville and Coles Creek periods, AD 700–1200, coincident with the beginning phases of the Mississippian tradition (Ford, 1952, Fig. 2). Krieger postulated that the Dunkin Incised cylinder jars found at the Davis site in eastern Texas may have their origin in Middle America (Krieger, 1949, p.223). Hall observed that the area occupied by the Caddoans “constituted a natural route for influences coming from Mexico toward the Cahokia area” (Hall, 2004, p. 101).

The Cahokia cylindrical vessel, like those at Chaco (Washburn, 2011), was decorated with a distinctive pattern and coloring system not found on local vessels from earlier periods (Fig. 3). To test the possibility that the Cahokia cylinder jar and associated vessel forms listed above contained cacao, we sampled 53 vessels from the monumental platform mound complex of Cahokia on the east side of the Mississippi river opposite present day St Louis (Fowler, 1997), sites adjacent to Cahokia in the American Bottom in the surrounding St Clair and Madison Counties, smaller “hinterland” platform mound sites and associated burial mounds—Dickson, Emmons, Frazier, Orendorf—located along bluffs above the Illinois River in Fulton County, Illinois (Fig. 4), and Late Mississippian sites in the lower Mississippi River Valley and Florida (Table 3).

Fig. 5 displays the rank ordering of the 47 Mississippian vessels containing measurable amounts of theobromine. We first established that this distribution is different from that expected from the geographical or dust controls. Fig. 6 compares the frequency of occurrence for the ranges of theobromine concentration from the Mississippian vessels to those of the geographical and dust controls. ANOVA revealed that theobromine [$F(2,74) = 15.8$; $p < .0001$] was significantly elevated relative to geographical controls ($p < .0001$) and relative to dust controls ($p < .0006$) (Table 4). This

Fig. 3. Cylinder jar, Cahokia, St. Clair County, Illinois. #73-2296. Courtesy, Cahokia Mounds State Historic Site.

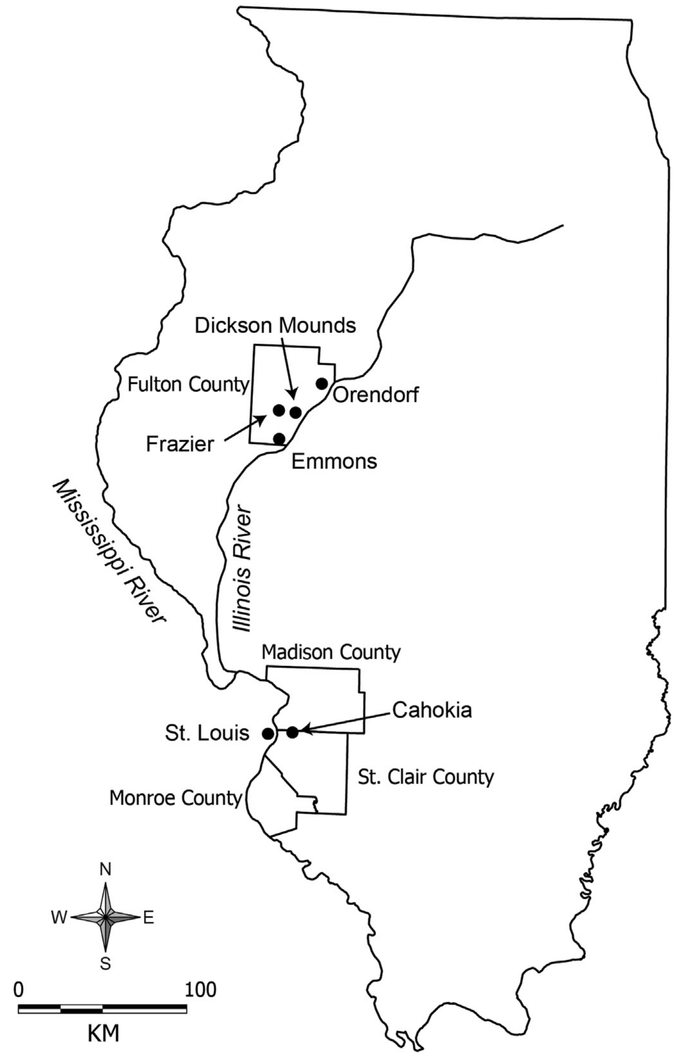

Fig. 4. The Middle Mississippi region locating Cahokia and the sites examined along the central, Illinois River Valley. Map by Michael Conner.

difference in theobromine levels is illustrated graphically in Fig. 7. These findings indicate that we can state with >99.5% confidence that the theobromine levels for the Mississippian ceramics as a group are significantly different from random environmental methylxanthine levels in airborne dust that contaminated the vessels during their curation and storage in a museum environment. This result mandates that there is a second source of the methylxanthines. We attribute this source to Mesoamerican cacao or Black Drink.

However, a similar analysis of the caffeine levels in the 46 vessels containing caffeine (Fig. 8) shows that the distribution of caffeine [$F(2,73) = 1.8$; $p = .17$] was not different from either of the controls, i.e., airborne contamination could account for the caffeine. In contrast, theophylline levels [$F(2,36) = 11.3$; $p < .0002$] in the 9 Mississippian vessels were different from the geographical controls ($p < .0001$) and from the dust controls ($p = .0002$), thereby again requiring a second source to be involved.

Although the ANOVA analysis enabled us to establish that there were two distinct populations of theobromine levels, statistics cannot identify which individual vessels contained levels of the theobromine sufficiently elevated to be indicative of the presence of cacao or Black Drink. However, we sought to identify the vessels with the highest probability of containing archaeological theobromine by employing a cutoff value based on the mean and standard deviation of the theobromine levels in the controls. The cutoff value is the minimum concentration of theobromine indicative of an additional source besides airborne contamination. We propose that the vessels with the greater probability of containing archaeological theobromine would be those with concentrations greater than this cutoff value.

To be conservative we elected to require the cutoff value for the theobromine level in the Mississippian vessels to be three standard deviations beyond the mean of the control group. For the dust samples, the sum of the mean and three standard deviations is 37 ng/mL; for the geographical controls, that value is 51 ng/mL. We used the higher cutoff value from the geographical controls. This value is indicated by the heavy horizontal line in Fig. 5. By this criterion, theobromine concentrations for 24 of the 47 vessels containing theobromine required exposure to cacao or Black Drink (*I. vomitoria*). Of these 24 vessels, 6 vessels contained more caffeine than theobromine consistent with Black Drink, 17 contained more theobromine consistent with cacao, and one contained equal amounts of the two methylxanthines and thus cannot be assigned to either Black Drink or cacao. Similarly utilizing the three standard deviation cutoff requirement for theophylline (>19 ng/mL), 3 vessels contained elevated theophylline levels and so suggests exposure to cacao since *I. vomitora* does not contain theophylline. In summary, 18 vessels of the 61 analyzed from the Midwestern sites appear to have been exposed to cacao.

Table 3
Data base of ceramics analyzed from sites in the Midwest/Southeast. $N = 53$.

Sample #	Catalog #	Vessel form	Museum	Site/provenience	Theobromine	Caffeine	Theophylline
201	E3.12 D119	Jar	D Mds	Dickson Md 10, B 81	307	116	24.9
202	E3.11 D270	Effigy bowl	D Mds	Dickson Md 10, B 168	121.4	96.4	0
203	E3.10 D181	Jar	D Mds	Dickson Md 10, B 94	111.8	63.4	0
204	F34 811.099	Necked bottle	D Mds	Dickson Md 11, B 399	16.6	8.4	0
205	11F0998-1003	Beaker	D Mds	Frazier Burial	0	16.1	0
206	E3.9 D121	Beaker	D Mds	Dickson Md 10, B 90	195.5	138.9	15.1
207	F34-47 D79	Beaker	D Mds	Dickson Md 10, B 47	167.3	98.5	22.4
208	E3.1 D389	Necked bottle	D Mds	Dickson Md 9, B 502	8.6	0	0
209	E4.10 D5	Jar	D Mds	Dickson Md 10, B 2	272.4	90.8	10.8
210	E5.11 D248	Jar	D Mds	Dickson Md 10, B 154	78.8	92.2	0
211	E7.14 811.010	Jar	D Mds	Dickson pre-Md cemetery, B 384	16	19	0
212	E8.6811.994	Bowl	D Mds	Dickson Md 6, B 786	18.6	19.1	0
213	E9.10 B630	Jar	D Mds	Dickson Md 5, B 630	22.8	27.9	0
214	F5.3812.439	Beaker	D Mds	Dickson Md 7, B 435	33.7	36.1	0
215	F5.2811.792	Bowl	D Mds	Dickson Md 8, B 658	40.7	33.6	0
216	F8.2812.213	Beaker	D Mds	Dickson Md 3, B 900	40.4	31.3	0
217	73-2296	Cylinder jar	Cahokia	E Stockade, Cahokia	13.6	16.7	0
218	2011-64-01	Bowl	Cahokia	Madison Cty	115.8	114.8	0
219	2011-64-05	Jar	Cahokia	St Clair Cty	22.6	17.3	26.3
220	2011-64-03	Jar	Cahokia	Madison Cty	54.8	6.4	0
221	2011-64-11	Bowl	Cahokia	St Clair Cty	10.7	6.5	0
222	2011-64-12	Jar	Cahokia	St Clair Cty.	13.6	12.5	0
223	89-01-102	Necked bottle	Cahokia	St Clair Cty	70.6	55.3	8.7
224	MS-38/217	Jar	Cahokia	Monks Md F 20, Cahokia	67.3	53.3	0
225	800-842	Necked bottle	Cahokia	Madison Cty	16.7	4.3	0
226	89-01-130	Bowl	Cahokia	E Stockade H 4, Cahokia	50.8	111.9	0
227	800-357	Duck effigy	Cahokia	Madison Cty	11.2	13.7	0
228	800-358	Mussel effigy	Cahokia	Madison Cty	10.7	25.8	0
229	11S34 371-19-1	Jar	Cahokia	Interp center, Cahokia	0	2.3	0
230	67-197-14	Bowl	Cahokia	E Stockade H 4, Cahokia	14.4	25.1	0
231	1993-0072-824399	Beaker	Ill St Mus	Emmons	21.8	7.9	0
232	1993-0072-824409	Beaker	Ill St Mus	Emmons Md A, B 4	16.1	6.3	0
233	1993-0072-824390	Beaker	Ill St Mus	Emmons Md A, B 12	76.2	22	0
234	1993-0072-824407	Beaker	Ill St Mus	Emmons	9.6	2.5	11.5
236	1993-0072-824375	Beaker	Ill St Mus	Emmons Md 58, B 42	103.1	35.3	7.3
237	1993-0072-824387	Beaker	Ill St Mus	Emmons Md 58, B 33	60.3	48.8	0
238	1993-0072-824374	Beaker	Ill St Mus	Emmons Md 58, B 28	51.4	38.2	0
239	1993-0072-824404	Beaker	Ill St Mus	Emmons Md 58, B 10	19.8	3.6	0
240	1111-05-8-802756	Bowl	Ill St Mus	Mack Bayou, Fla.	78.6	96.2	0
244	2000-0079-000002	Owl effigy	Ill St Mus	Orendorf, Ill.	0	0	0
246	1111-0508-802758	Bowl	Ill St Mus	Mack Bayou, Fla.	96	119.2	0
247	1111-0508-802757	Bowl	Ill St Mus	Mack Bayou, Fla.	53.9	85.2	0
248	1993-0072-824378	Necked bottle	Ill St Mus	Emmons Md 58, B 32	52.3	90.6	0
250	1993-0072-824406	Jar	Ill St Mus	Emmons Md 58, B6	158.9	64.2	12.8
253	1993-0072-824329	Duck effigy	Ill St Mus	Emmons Md A, B 6	65.4	29.7	0
254	1993-0072-824307	Jar	Ill St Mus	Emmons Md 58, B 9	118.5	11.6	0
256	1993-0072-824311	Bowl	Ill St Mus	Emmons Md 58, B 22	248.4	31.2	0
259	79-4-10/19665	Bowl	Peabody	Mooses Farm, Ark.	0	0	0
260	80-20-10/22060	Necked bottle	Peabody	Rose Md, Ark.	0	0	0
262	01-16-10/56970.1	Cylinder jar	Peabody	Pemiscot Md, Ark.	9.7	4.3	0
263	80-20-10/21816	Necked jar	Peabody	Pemiscot Md, Ark.	7.9	0	0
264	01-16-10/56991	Stirrup bottle	Peabody	Neeleys Ferry, Ark.	0	0	11.8
265	02-19-10/64293	Necked bottle	Peabody	Edwards Md, Miss.	9.8	0	0

9.2. *Pueblo Bonito and Los Muertos reanalysis*

Having established that methylxanthine contamination was a confounding factor, it was incumbent on us to revisit our original analyses of Pueblo Bonito ceramics (Washburn et al., 2011). We employed ANOVA statistics in conjunction with the geographical and dust controls to distinguish vessels from the Southwest containing methylxanthines that were residues from prehistoric consumption of cacao from those that only contained airborne methylxanthines.

We restricted this reanalysis to ceramics from Pueblo Bonito, as well as Aztec Ruins, a great house "outlier" on the San Juan River to the north of Chaco Canyon, Pueblo del Arroyo, a great house along the Chaco Wash in Chaco Canyon, and BC-51, a small masonry unit-pueblo site on the south side of the Chaco Wash occupied contemporaneously with the great houses (Table 5). The three vessels from Pueblo Del Arroyo included a cylinder jar with a design also found on cylinder jars from Pueblo Bonito and two "vases"—slope-sided red painted and polished cylinder jar-like forms unique to Chaco (Judd, 1954, Pl.55). Of the three vessels sampled from BC-51, one was a local globular pitcher form decorated with the local design system and two were large bowls decorated with hatched designs of the new Chaco design system. We analyzed 60 vessels, 36 of which contained measurable amounts of theobromine.

For Pueblo Bonito ANOVA analyses revealed significant elevations in samples from archaeological vessels relative to environmental controls for theobromine and theophylline. Theobromine [$F(2,67 = 7.7)$; $p < .001$] was elevated relative to geographical controls ($p < .0001$) and the dust controls ($p < .0006$) (Fig. 9).

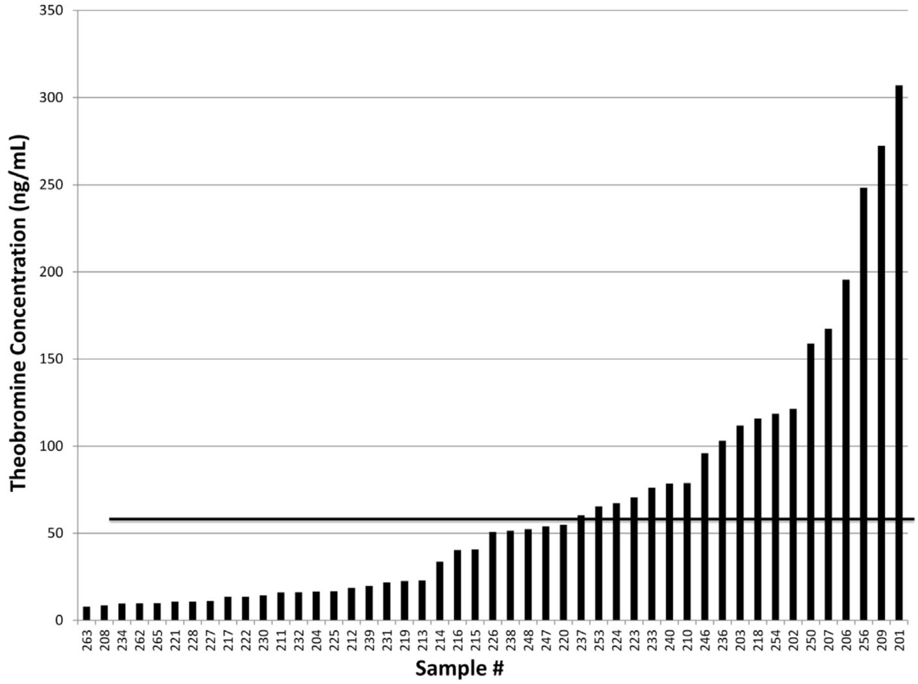

Fig. 5. Rank order of the 47 Mississippian period vessels from the Midwest/Southeast containing theobromine. The heavy horizontal line indicates theobromine concentration three standard deviations above the mean airborne contamination.

Theophylline was also elevated relative to geographical controls ($p < .0001$) and dust controls ($p < .0002$). Caffeine was not altered relative to controls in this analysis. Applying the three standard deviation cutoff requirement of 51 ng/mL for theobromine containing vessels, 12, 20% of the 60 analyzed vessels contained sufficiently elevated theobromine levels suggestive of cacao residues (Fig. 10). Of these twelve vessels, the theophylline concentration for five also exceeded the cutoff value further bolstering the presence of cacao.

In addition, we reanalyzed our original analysis (Washburn et al., 2011) of 10 vessels from Los Muertos, a large late Classic platform mound site of the Hohokam tradition located south of the Salt River in southern Arizona (Table 6). We had reported that 8 of 10 vessels contained detectable amounts of theobromine. Since all of these samples were housed in the same museum (Peabody), statistical analysis was based on the 10 dust samples from the Peabody. Theobromine [$F(2,32 = 4.2)$; $p = .024$] was elevated relative to the geographical controls ($p < .0008$) but was not elevated relative to the dust controls because of the small sample sizes. In contrast, the levels of caffeine [$F(2,34 = 0.22)$; $p = .80$] and theophylline [$F(2,32 = 0.11)$; $p = .11$] can be accounted for by airborne contamination. Applying the three standard deviation cutoff requirement of 51 ng/mL of theobromine containing vessels, we find that 3 vessels, 30% of the 10 analyzed vessels, contained sufficiently elevated measurable levels of theobromine indicative of cacao consumption.

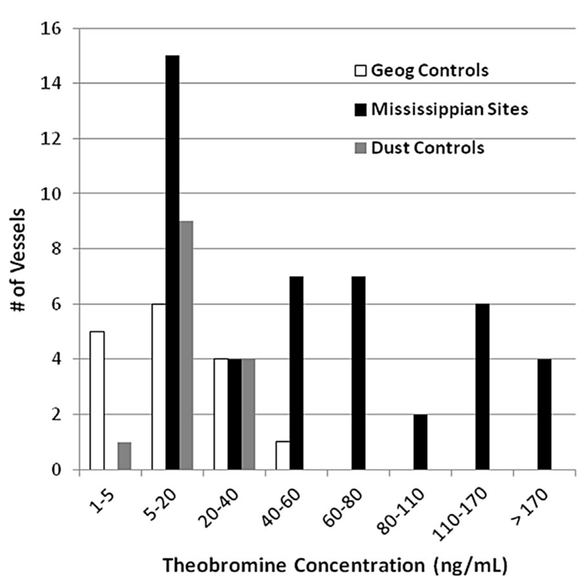

Fig. 6. Distribution of theobromine levels in 47 Midwest/Southeast Mississippian vessels compared to theobromine levels in the geographical and dust controls.

9.3. Abajo R/O reanalysis

The Abajo results are an enigma. In our prior report of the Abajo vessels from Alkali Ridge (Washburn et al., 2013) we detected low levels of theobromine and caffeine. These levels are consistent with the levels we found with the dust contamination controls. The failure to detect any theophylline is perplexing since all three methylxanthines were present in all 10 dust samples taken from the shelves of the Peabody storage facility where the Abajo vessels were stored. Thus, it is difficult to ascribe the theobromine and caffeine detected in these vessels to dust contamination since theophylline should also have been detected. However, two factors may explain the failure to detect theophylline. First, when we tested them, the bowls were unusually clean with no obvious dust. Since they were unusual finds, they may have been well cleaned for study. In addition, they are stored upside down in the drawers, precluding dust settling in the interior. For these reasons we suggest that the caffeine and theobromine levels detected are actually from the archaeological record and not due to airborne contamination. Since theophylline is much less prevalent than theobromine in *T. cacao*, if only low levels of theobromine remain, the

Table 4
Summary of statistics for methylxanthine levels in vessels from the areas analyzed.

Methylxanthine	No. of vessels w/methylxanthine	Mean concentration ± SEM (ng/mL)	*p* vs dust controls	*p* vs. geographical controls
***Mississippian n* = 61**				
Theobromine	47	67.7 ± 10.5	<.0004	<.0002
Caffeine	46	45.5 ± 5.9	*	*
Theophylline	9	15.5 ± 2.4	.0001	.0001
***Pueblo Bonito n* = 60**				
Theobromine	36	60.8 ± 14.6	<.02	< .02
Caffeine	33	163.7 ± 42.3	*	.02
Theophylline	20	24.9 ± 5.2	<.0008	<.0004
***Los Muertos n* = 10**				
Theobromine	8	59.4 ± 19.9	*	.0008
Caffeine	9	58.0 ± 25.1	*	*
Theophylline	8	9.2 ± 2.3	*	*

*Not significant, $p > .05$.

theophylline level would fall below detection limits, thus accounting for the failure to detect theophylline. In summary, these findings support our contention that cacao was being imported and consumed as early as the 8th century.

10. Insights from cacao use in the American Midwest and Southeast

In a recent review article White and Weinstein concluded that "Mississippian culture [is] an indigenous development not derived from Mesoamerican origins" (2008; p. 259). Noting that "the greatest mystery to an archaeologist is the absence of any prehistoric alcoholic drink in the Southeast" (and indeed most of North America north of Mexico), they also remark that a popular Mexican drink, cacao, is "another missing element in the Southeast" (2008; pp. 254–255). In this paper we have resolved their mystery regarding cacao.

We suggest that the cacao arrived with other goods along an active trade network along the Gulf coast. The symmetrical organization of the Dunkin Incised, AD 900–1300, designs on the cylinder jars from the Caddo area (Newell and Krieger, 1949, Fig. 41H, Townsend and Walker, 2004, Fig. 6, right) suggests that the designs share affinities with woven structures on basketry (Holmes, 1896, Figs. 14 and 22; Townsend and Walker, 2004, p. 236). It is likely that goods such as cacao beans arrived in the Southeast in baskets. Their fabric structures may have inspired the incised designs on the Caddo cylinder jars. Certainly, light, serviceable baskets would have been preferable to heavier ceramics for carrying goods over long distances. We suggest that cacao as well as native tobacco (Emerson, 2003, p. 143) and other plant products may have been part of a widespread trade network during the late prehistoric period. Johannessen, for example, has reported Prickly mallow seeds (*Sida spinosa*) from three Late Woodland and Mississippian period features at the BBB motor site, a Mississippian site near Cahokia (1984a, p.182). This plant is native to the US Gulf coast states, the north and east coasts of Mexico, the Caribbean and Mesoamerica, but not to Illinois or Missouri where they were found at the BBB site (www.ars-grin.gov/cgi-bin/npgs).

Here we comment on how the cacao evidence can illuminate several archaeological issues.

10.1. Cahokia/Hinterland site relationships

We tested 5 vessels from the main Mississippian site of Cahokia, 2 of which—a shouldered jar from Monks Mound, Feature 20 (sample 224), and a bowl from House 4 in the East Stockade (sample 226) – contained theobromine levels statistically above geographical control levels; ie., not due to airborne contamination. Statistical analysis will not rule out the caffeine levels being due to airborne contamination. That said, the caffeine level for sample 226 is double the level for theobromine as would be expected if it contained Black Drink. In contrast, the caffeine level for sample 224 is less than theobromine as would be expected if it contained cacao. Both vessels were red slipped and dated to the Stirling Phase at the height of the Mississippian occupation in the American Bottom region.

In addition we tested vessels from long destroyed mound complexes located in Madison and St Clair Counties surrounding

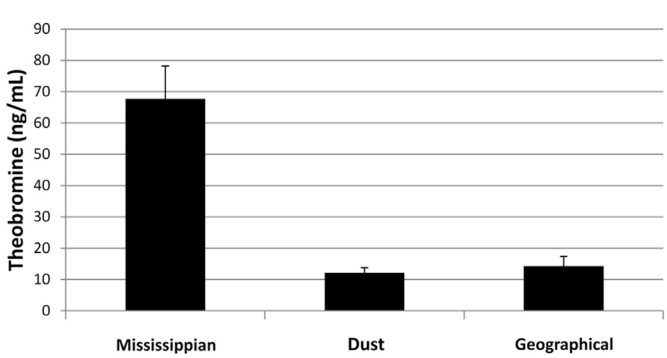

Fig. 7. Mean values of the theobromine concentrations in the Midwest/Southeast vessels and the geographical and dust samples with one standard deviation of the mean indicated.

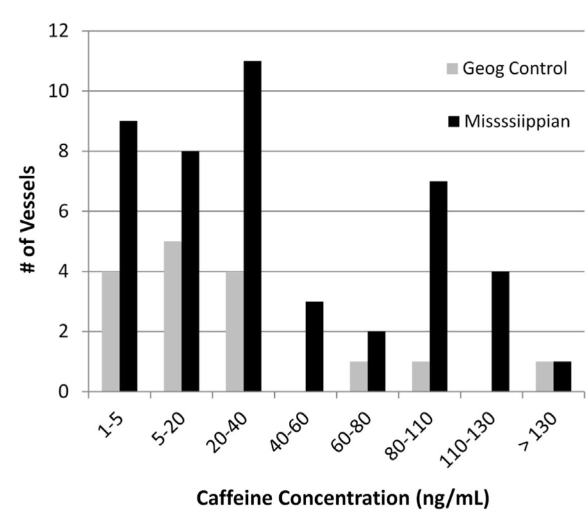

Fig. 8. Distribution of caffeine levels in 46 Midwest/Southeast Mississippian vessels compared to caffeine levels in the geographical controls.

Table 5
Data base of ceramics reanalyzed from Chaco Canyon. $N = 60$.

Sample #	Catalog #	Vessel form	Museum	Site	Room	Theobromine	Caffeine	Theophylline
1	H/3263	Cylinder jar	AMNH	Bonito	28	6.8	0	0
2	H/3231	Cylinder jar	AMNH	Bonito	28	5.8	0	0
3	H/3251	Cylinder jar	AMNH	Bonito	28	15.5	0	0
4	H/3262	Cylinder jar	AMNH	Bonito	28	186.78	1070	8.5
5	H/3261	Cylinder jar	AMNH	Bonito	28	0	0	0
6	29.1-3215	Pitcher	AMNH	Aztec		0	0	0
7	H/3269	Pitcher	AMNH	Bonito	28	46.99	410	5.8
8	H/4153	Cylinder jar	AMNH	Bonito	28	0	0	0
9	H/3284	Pitcher	AMNH	Bonito	28	0	0	0
10	H/3266	Cylinder jar	AMNH	Bonito	28	12.8	0	0
11	H/3225	Cylinder jar	AMNH	Bonito	28	11.2	0	0
12	H/3252	Cylinder jar	AMNH	Bonito	28	0	0	0
13	H/3576	Pitcher	AMNH	Bonito	32	0	0	0
14	H/3271	Pitcher	AMNH	Bonito	28	57.11	510	5.3
15	H/3236	Cylinder jar	AMNH	Bonito	28	0	0	0
16	H/3243	Cylinder jar	AMNH	Bonito	28	334.92	680	10.3
17	H/3229	Cylinder jar	AMNH	Bonito	28	0	0	0
90	H/3199	Bowl	AMNH	Bonito	28	48.2	371.7	0
91	H/3675	Bowl	AMNH	Bonito	33	0	0	0
92	H/3879	Bowl	AMNH	Bonito	2	14.7	98.5	0
93	H/3198	Bowl	AMNH	Bonito	28	0	0	0
94	H/3589	Bowl	AMNH	Bonito	32	6.6	50	0
95	H/3598	Bowl	AMNH	Bonito	32	36	87.4	0
96	H/3586	Cylinder jar	AMNH	Bonito	32	4.5	26.3	0
97	H/3595	Cylinder jar	AMNH	Bonito	32	0	0	0
98	H/5223	Bowl	AMNH	Bonito	38	14.6	15.9	0
99	H/3584	Pitcher	AMNH	Bonito	32	0	0	0
100	H/3585	Pitcher	AMNH	Bonito	32	6	24.1	0
101	H/3615	Pitcher	AMNH	Bonito	32	4.6	172.9	0
102	H/3616	Pitcher	AMNH	Bonito	32	0	0	0
103	H/3633	Pitcher	AMNH	Bonito	32	7.8	13.3	0
104	H/3676	Pitcher	AMNH	Bonito	33	0	23.9	0
105	H/5619	Pitcher	AMNH	Bonito	53	0	0	0
106	H/3592	Pitcher	AMNH	Bonito	32	3.6	22.5	0
107	H/3287	Pitcher	AMNH	Bonito	28	0	0	0
108	H/3619	Pitcher	AMNH	Bonito	33	0	0	0
109	H/3275	Pitcher	AMNH	Bonito	28	0	25.5	0
110	H/3276	Pitcher	AMNH	Bonito	28	0	0	0
70	A336410	Pitcher	Smithsonian	Bonito	320	0	0	0
71	A336339	Bowl	Smithsonian	Bonito	330	37.4	35.9	22
72	A336433	Pitcher	Smithsonian	Bonito	326	0	0	0
73	A336407	Pitcher	Smithsonian	Bonito	320	24.7	36.2	5
74	A336226	Bowl	Smithsonian	Bonito	323	79.1	68.3	52
75	A336501	Cylinder jar	Smithsonian	Bonito	330	395.3	584.8	75
76	A336492	Cylinder jar	Smithsonian	Bonito	326	83.3	31.7	0
77	A336177	Bowl	Smithsonian	Bonito	266	29.2	18.4	9
78	A336469	Pitcher	Smithsonian	Bonito	330	33.3	58.1	26
79	A336344	Bowl	Smithsonian	Bonito	330	34	47.6	11
80	A336496	Cylinder jar	Smithsonian	Bonito	329	94.9	229.2	43
81	A334575	Cylinder jar	Smithsonian	Del Arroyo	15	117.2	116.5	61
82	A334577	Cylinder jar	Smithsonian	Del Arroyo	15	161.8	321.3	85
83	A334576	Cylinder jar	Smithsonian	Del Arroyo	15	80.2	94.4	18
40	30-18-10/A6918	Cylinder jar	Peabody	Bonito	28	0	0	0
41	30-18-10/A6921	Cylinder jar	Peabody	Bonito	28	0	0	0
42	30-18-10/A6919	Cylinder jar	Peabody	Bonito	28	21.6	70.5	12
54	30-18-10/A6916	Pitcher	Peabody	Bonito	28	0	0	0
55	30-18-10/A6917	Pitcher	Peabody	Bonito	28	14.7	25	13
52	39-79-10/18676	Bowl	Peabody	BC-51		7.5	8.6	8
53	39-79-10/18679	Bowl	Peabody	BC-51		98.4	41.8	22
56	39-79-10/18675	Pitcher	Peabody	BC-51		51.3	11.2	6

Cahokia. Two vessels (samples 218 and 220) from sites in Madison Country and one vessel (sample 223) from a site in St Clair County contained theobromine in levels above airborne contamination. With the same provisos regarding caffeine stated above, two vessels contained more theobromine than caffeine (samples 220 and 223), suggestive of cacao consumption. These sites were dated to the Moorehead Phase that marked the beginning of the decline of the Mississippian occupation at Cahokia.

We tested 2 conch shells from the Cahokia area (Samples 257 and 258). Since historic records indicate that such shells were used for black drink consumption (Milanich, 1979), we wondered whether they were used in the earlier Mississippian period to consume beverages. However, we were unable to detect any methylxanthines in these samples, possibly because the calcium carbonate of conch shell is alkaline, an unfavorable environment for adherence and penetration of methylxanthines. Thus, if cacao or Black Drink were consumed in these shells, any surface residues could be easily lost to cleaning. In contrast, methylxanthines adhere more tightly to acidic cations that are present in the clay matrix of ceramics.

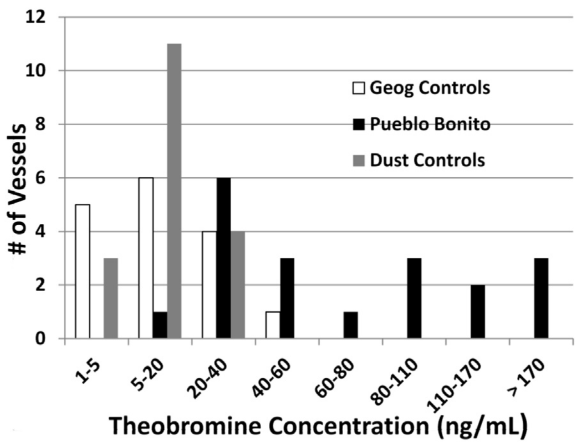

Fig. 9. Distribution of theobromine levels in 36 Chaco vessels compared to theobromine levels in the geographical and dust controls.

We also inquired whether cacao was consumed at the hinterland platform mound complexes in order to clarify the nature of the affiliation of these sites with activities and personnel at the central site of Cahokia. Elite presence at the hinterland sites is indicated in some burial assemblages, some of which have striking similarities to burials at Cahokia. For example, in the Dickson Mound E burials, there was a high incidence of elite regalia as well as Ramey Incised and Powell Plain vessels (Harn, 1980, p. 78). In Dickson Mound B the side by side burial of four decapitated males recalls a similar burial at Cahokia Mound 72 said to be that of sacrificial retainers in which four males were buried without their heads and hands. In the Dickson Mound B burial Mississippian and Woodland vessels that included an owl effigy hooded water bottle with Ramey Incised scroll motifs (Harn, 1975, p. 428), a Sepo Smoothed-over Cordmarked jar, a plain jar and Mossville Cordmarked jars (Harn, 2011) were substituted for the heads. A high status burial (#31) from the Emmons cemetery included a number of rare objects: a copper covered red cedar mask, ear discs, conch shell pendants, pottery vessels and lithics (Morse et al., 1961).

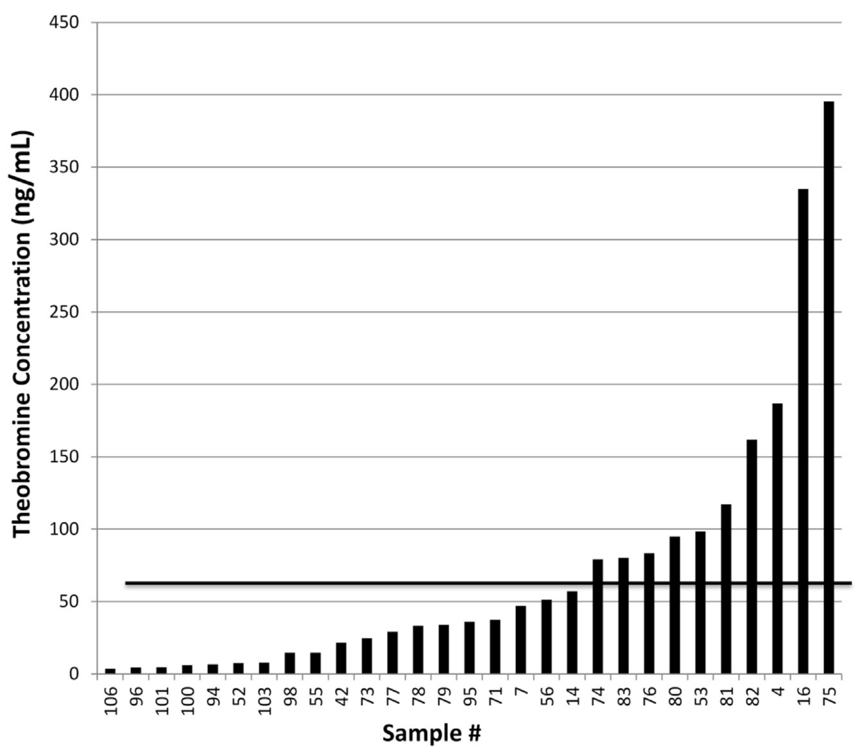

Fig. 10. Rank order of the 36 vessels from Chaco Canyon containing theobromine. The heavy horizontal line indicates theobromine concentration three standard deviations above the mean airborne contamination.

Table 6
Data base of ceramics reanalyzed from Los Muertos. $N = 10$.

Sample #	Catalog #	Vessel form	Provenience	Theobromine	Caffeine	Theophylline
48	46-73-10/55275	Pitcher	Refuse Md 19a	156	12.8	3.3
49	46-73-10/55061	Pitcher	Refuse Md 2a	8.7	0.1	3.3
50	46-73-10/55056	Cylinder	Refuse Md 14a	32	46.4	17
60	46-73-10/54945	Cylinder	Ruin 14	16.6	25.3	7
61	46-73-10/54948	Bowl	Ruin 19	46.2	81.7	19
62	46-73-10/54921	Mug	Refuse Md 14a	3.7	17.8	6
63	46-73-10/55001	Necked bottle	Refuse Md 14a	0	22.8	0
64	46-73-10/55041	Cylinder	Refuse Md 14a	94.9	267.8	14
65	46-73-10/54910	Pitcher	Ruin 13	116.7	88.4	4
66	46-73-10/54975	Cylinder	Ruin 20	0	16.7	0

Fig. 11. Vessels from the Dickson Mounds containing all three methylxanthines. Upper left #D5 jar (sample 209), burial 2; upper right, #D79 beaker (sample 207), burial 47; lower left, #D119 jar (sample 201), burial 81; lower right, D#121, beaker (sample 206), burial 90. Courtesy, Illinois State Museum.

The Dickson site consisted of at least 10 separate burial mounds and a small flat-topped pyramidal mound that was later used for burials. The mounds served as the cemetery for the nearby Eveland site whose circular sweat ledge, cross-shaped building, and two large rectangular structures indicate that it functioned as one of the hinterland centers (Harn, 1991). Late Woodland and Mississippian burial activity began at Dickson by AD 1100 with deceased from the Eveland site and continued until about AD 1200. Thereafter, burials at Dickson were of individuals from the Myer-Dickson site. Cessation of burial activity at Dickson AD 1260 coincided with the beginning of decline at Cahokia (Harn, 1980, p. 82).

We tested a total of 15 vessels (Fig.11) from six different burial mounds at Dickson, 7 of which were from Mound I (10), the largest burial mound in the Dickson complex. Although Harn concluded that the burials from Mound I "do not seem to be persons of unusual importance … no exotic ceremonial regalia or quantities of artifacts are represented which suggest superordinate social positions" (Harn, 1980, p. 29, Table 1), all 7 vessels from this area contained theobromine in levels three standard deviations above airborne levels, suggestive of cacao. In one of these burials, a skilled craftsman in Burial 90 (sample 206) was buried with two tool kits and a handled beaker. All burials are from the Larson Phase, AD 1300 coincident with the decline of Cahokia.

To confirm that the results from our study of vessels from the Dickson Mound hinterland complex were not an anomaly, we tested vessels from another hinterland platform mound complex, the Emmons site, that similarly encompassed burial mounds, village sites and a cemetery (Morse et al., 1961, Table 1) (Fig. 12). We

Fig. 12. Vessels from the Emmons Mound Cemetery containing all three methylxanthines. Left: beaker #1993-0072-824375 (sample 236), burial 42. Right: jar #1993-0072-824406 (sample 250), burial 6. Courtesy, Illinois State Museum.

tested 13 vessels, 9 of which contained theobromine in levels three standard deviations above airborne levels. Of the 9 vessels, 8 had more theobromine than caffeine, suggesting that they contained cacao, and, with the proviso that caffeine may be due to airborne contamination, one had more caffeine than theobromine suggesting it may have contained Black Drink. All these vessels were from burial contexts dating to the Larson Phase, again indicating burial use of a hinterland site at the end of Cahokia's hegemony when populations may have moved out of the center to these outlying areas.

10.2. Geographical and temporal range of cacao use

In this study we also initiated inquiries designed to establish the temporal and spatial boundaries of cacao use in the Midwest/Southeast. Due to the small number of samples analyzed, this initial inquiry suggests trends that require further analyses of larger samples. We tested 2 Late Woodland Sept cordmarked jars from burials in the Dickson Mounds to ascertain whether cacao use was practiced prior to the Mississippian period. These 2 vessels (samples 211 and 213) did not contain methylxanthines above airborne contamination levels.

We inquired whether consumption of cacao extended into the post-Cahokia Late Mississippian period in sites along the southern Mississippi River and its tributaries as well as further east in Florida. We sampled 9 vessels from sites in Arkansas, Mississippi and Florida. The Edwards Mound, a group of large rectangular platform mounds on the Sunflower River in Coahoma County, Mississippi was excavated by Charles Peabody (Peabody, 1904) and later relabeled the Oliver site (16-N-6) by Phillip Phillips (Phillips et al., 1951, p. 253). Six vessels were sampled from mound sites in Cross County, northeast Arkansas known by several names and site codes (Morse, 1981, 1990; Phillips et al., 1951): Rose Mound (3CS27, 12-N-3), Vernon-Paul (Pemiscot, 3CS25, 11-N-9) and Neeley's Ferry (3CS24, 11-N-4). Characterized as “St Francis type” sites (Phillips et al., 1951, p. 329), that is, planned villages oriented to the cardinal directions, they include a platform mound, habitation houses around the edges of the plaza all of which are surrounded by a moat or palisade. Calibrated dates from Vernon-Paul range from AD 1280–1475 (Gannon, 2002, p. 20). Neeley's Ferry is slightly later, AD 1400–1600, but both sites are within the Parkin Phase (Phillips, 1970) of the Late Mississippian period (Brown, 2005). The methylxanthines found in the Mississippi and Arkansas vessels were not above airborne levels. Regarding the vessels from the Florida site of Mack Bayou, we cannot rule out that the caffeine present was not due to airborne contamination. However, if it was not due to contamination, these three vessels probably contained Black Drink (samples 240, 246, and 247). These bowls have a carinated vessel form and are decorated with incisions and punctations typical of Mississippian vessel shapes and decorations (Marrinan and White, 2007; p. 293).

11. Insights from cacao use in the American Southwest

The discovery of the presence of theobromine as a residue in a vessel form, the cylinder jar, new to the Southwest (Crown and Hurst, 2009) as well as one decorated with a non-local design system (Washburn, 2011), prompted us to explore a more robust sample that would indicate the nature and extent of the use of cacao in the American Southwest Washburn et al., 2011). We proposed that the presence of cacao consumption resulted from trade for precious materials such as high grade turquoise found especially in the Southwest that were purchased with cacao money.

In our reanalysis of this initial research with contamination considerations in mind, we detected theobromine in levels consistent with cacao usage in 7 vessels from Pueblo Bonito, 3 vessels from Pueblo del Arroyo and 2 vessels from BC-51. In assessing the relatively low frequency of methylxanthines from Pueblo Bonito, it is important to consider the context in which the vessels from Pueblo Bonito were found. Of the 25 vessels sampled from Room 28, only 3 had significant levels of theobromine beyond that of dust contamination. Many vessels from Room 28 had no detectable levels of any methylxanthines, suggesting that many had been made but never used. It is well known that Room 28 functioned as a storeroom, housing a pile of cylinder jars stacked like cordwood (Pepper, 1920, Fig. 43). The fact that vessels in this pile had no detectable methylxanthines suggests that this pile represents jars in various stages of manufacture, painting, and use (see also Crown and Wills, 2003). In fact, the two plain white cylinder jars tested from Room 28 (samples 5 and 10) had no measurable amounts of methylxanthines, suggesting that they represented vessels that had been made and slipped but not yet decorated. In contrast, of the 9 vessels from Rooms 320, 326, 329, and 330 tested, 4 had significant levels of theobromine. Their association with richly appointed burials (Judd, 1954; p. 325ff) suggests that either these vessels belonged to the individuals buried and/or that cacao drinks were included as offerings when the individuals were buried. Significantly, both red vases from Pueblo Del Arroyo contained substantial amounts of all three methylxanthines. Given that the form and red surface finish of these jars is atypical for the Chaco area coupled with the evidence that they contained cacao, we propose that they were brought into Chaco, perhaps even from the cacao source area, as models for use in the cacao processing activities. We also reanalyzed our data from the Hohokam site of Los Muertos and found that 3 of the 10 vessels, a cylinder jar and two pitchers, had statistically significant levels of theobromine, indicating that cacao consumption was also taking place at large platform mound sites in southern Arizona.

12. Conclusion

This article has focused on the controls and statistical analyses needed to conduct residue analyses to detect methylxanthines. Since methylxanthines are known to be in airborne particulate matter, we assessed the presence of this contamination on artifacts in museum storage environments. We ran control assays of vessels from areas and periods unlikely to have had access to cacao and of dust samples from six different museum laboratories and storage rooms where the vessels tested were stored. Our research indicates that a simple detection of methylxanthines cannot be taken as unequivocal evidence that cacao was processed and consumed in the archaeological situation under study.

In light of the pervasive presence of methylxanthines as airborne particulate matter detected in our dust controls that also settles on and thus contaminates the surface of artifacts, it is necessary to reconsider how this finding affects the different methods used to collect samples of absorbed residues. For the methylxantines, airborne contamination will always be an issue for the water washing procedure. In addition, analyses of residues scraped from the surface may also be confounded by airborne contamination, although no controls have been published indicating whether scraped residues contain ambient dust contaminants.

Depending on the depth and extent of surface removal, contamination may also be a problem for the burring and extraction of pulverized materials collected from vessels and sherds which has been the principal method of sample collection. Only future control studies will resolve whether the latter method avoids surface contaminants that otherwise would produce false positives. This possibility is greatly increased when analysis of the

pulverized method yields very low levels of methylxanthine of <1 ng/mL since failure to burr sufficiently deeply to remove any contaminating dust imbedded in the crevices of the clay matrix may generate these levels (see, for example Table 2, Crown et al., 2012).

Museum curators and conservators should take note that while we have established that this issue of airborne contamination is a particular problem when conducting studies to detect the methylxanthines, archaeologists who study and sample collections stored under current conditions should be forewarned that there are many other compounds that may be absorbed as contaminants on airborne particulates, only a few of which have been identified. Ubiquitous smoking by researchers in the field and laboratory may contaminate artifacts subjected to analyses for nicotine. Likewise, the frying activities of fast food establishments and use of biodiesel must surely contaminate the air with fatty acids. Since these compounds are ubiquitous in foods, testing of surface and absorbed residues for fatty acids may be compromised by such airborne particulates. Thus, when testing for a particular compound of interest, controls should be used to eliminate the possibility that such compounds are included in airborne particulate matter. In addition attention should be given to field collection and sampling techniques since certain procedures may compromise the analyses (see, for example, Lovis' discussion of curatorial concerns when dating residues adhering to artifacts, 1990).

However, despite the confounding challenge of methylxanthines as airborne contaminants of vessels in museum storage environments, our studies of large samples provide unequivocable evidence of vessels containing cacao from the American Midwest/Southeast and Southwest. We argue that by analyzing robust sample sizes, performing the requisite controls to eliminate alternative sources and using statistical analyses to verify the results, it is possible to differentiate vessels with low levels of airborne methylxanthine contaminants from vessels with higher levels of methylxanthines indicative of actual prehistoric use of cacao and/or Black Drink. Despite having employed highly conservative criteria entailing a 3 standard deviation separation (99.5% probability) for identification of cacao containing vessels, 20–30% of the Southwestern and 37% of the Midwestern vessels were deemed to have contained cacao. Relaxation of the cutoff value would substantially increase these percentages. Given this evidence of cacao use in both the American Southwest and Southeast/Midwest, we suggest that attention should now turn to the nature of interaction and exchange between these areas and Mesoamerica, the source area for the cacao and the many cultural uses and practices associated with this plant.

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